In the Claims

Please add new claim 89 as indicated in the listing of claims below pursuant to 37 C.F.R. §1.121.

1. (Original) Isolated nuclear protein which binds, in a sequence specific manner, to a transcriptional regulatory DNA element of an immunoglobulin light chain genes, a transcriptional regulatory DNA element of an immunoglobulin heavy chain genes or both.

2. (Original) Isolated nuclear protein, which binds, in a sequence specific manner, to enhancer DNA sequences of the kappa light chain gene.

3. (Original) A nuclear protein of claim 2, wherein the sequences are TGGGGATTCCCA.

4. (Original) Isolated NF-κB.

5. (Original) Isolated nuclear protein, which:
   a) binds to DNA sequences in the upstream region of both mouse heavy and Kappa light chain gene promoters; and
   b) binds to DNA sequences of mouse heavy chain gene enhancer.

6. (Original) A nuclear protein of Claim 5, wherein the sequences are ATTTGCAT.

7. (Original) Isolated nucleic acid encoding a nuclear protein of Claim 1.

8. (Original) Isolated nucleic acid encoding a nuclear protein which binds, in a sequence specific manner, to enhancer DNA

sequences of the Kappa light chain gene.

9. (Original) Isolated nucleic acid of Claim 8 wherein the nuclear protein binds, in a sequence specific manner , to enhancer DNA sequences of the Kappa light chain gene.

10. (Original) Isolated DNA encoding a structural gene for a nuclear protein, which protein binds in a sequence specific manner to the kappa enhancer.

11. (Original) Isolated DNA which encodes a nuclear protein which:
    a) binds to DNA sequences in the upstream region of both mouse heavy and Kappa light chain gene promoters; and
    b) binds to DNA sequences of mouse heavy chain gene enhancer.

12. (Original) DNA encoding the transcriptional regulatory factor IgNF-B (NF-A2).

13. (Original) A cloned DNA sequence which encodes a protein which binds to the κ-element TGGGGATTCCCCA and which hybridizes to a single, approximate 10kb RNA transcript from both B and non-B human cells.

14. (Original) An assay for detection of binding of cellular nuclear protein to DNA, comprising the steps of:
    a) providing an extract of cellular nuclear protein;
    b) preparing an incubation mixture consisting of:
        i) the extract of nuclear protein;
        ii) a radiolabeled DNA fragment to be tested for binding with the nuclear protein; and
        iii) an alternating copolymer duplex poly(dI-dc)-

poly(dI-dc);

c) incubating the mixture under conditions which allow the formation of protein-DNA complexes; and

d) resolving complexed DNA from free DNA by electrophoresis through a low ionic strength, nondenaturing polyacrylamide gel.

15. (Original) An assay of Claim 14, wherein the radiolabeled DNA fragment to be tested is less than about 100 base pairs.

16. (Original) A method of Claim 14, wherein the DNA fragment is end-labeled with ^{32}P.

17. (Original) A method of enhancing the transcription of a gene of interest whose transcription is regulated by a regulatory factor which binds DNA in the vicinity of the gene, comprising the steps of:

a) preparing an expressible gene construct comprising a strong promoter linked to a gene encoding the regulatory factor; and

b) incorporating into a cell containing the gene of interest, single or multiple copies of the gene construct sufficient to enhance transcription of the gene of interest.

18. (Original) A method of Claim 17, wherein the cell is a lymphoid cell and the gene of interest is a gene encoding an Ig chain.

19. (Original) A method of Claim 17, wherein the regulatory factor is selected from the group consisting of the following factors: IgNF-A, E, IgNFB and NF-κB.

20. (Original) A method of Claim 17, wherein the regulatory factor is IgNF-B.

21. (Original) A method of Claim 17, wherein the lymphoid cell is a hybridoma cell.

22. (Original) A method of enhancing transcription of a gene encoding an Ig chain, comprising:

a) preparing an expressible gene construct comprising a strong promoter linked to DNA encoding a structural gene for B-cell nuclear protein, which protein binds in a sequence specific manner to a transcriptional regulatory DNA element of an immunoglobulin light chain genes, a transcriptional regulatory DNA element of an immunoglobulin heavy chain gene or both; and

b) transferring an Ig chain-producing lymphoid cell with the construct in multiple copies to enhance the transcription of the Ig chain-encoding gene.

23. (Original) A method of claim 22, wherein the DNA encoding the structural gene for a B-cell nuclear protein encodes IgNFB.

24. (Original) A lymphoid cell transformed with an expressible nucleic acid construct comprising nucleic acid encoding a transcriptional regulatory factor which regulates Ig gene transcription.

25. (Original) A lymphoid cell of claim 24, which is a hybridoma.

26. (Original) A lymphoid cell of claim 24, wherein the regulatory factor is B-cell nuclear protein, which protein

binds in a sequence specific manner to a transcriptional regulatory DNA element of an immunoglobulin light chain gene, a transcriptional regulatory element of an immunoglobulin heavy chain gene, or both.

27. (Original) A lymphoid cell of claim 26, wherein the regulatory factor is IgNF-B or NF-κB.

28. (Original) A method of screening for the expression of a sequence-specific binding protein by a recombinant expression vector, comprising contacting protein produced by a host cell transformed by the recombinant vector with a nucleic acid recognition site probe, under conditions which permit the specific formation of a complex of the sequence-specific binding protein and the recognition site probe and determining whether such formation of a complex occurs, wherein formation of a complex is an indication of the expression of the sequence-specific binding protein by the recombinant vector.

29. (Original) A method of identifying recombinant expression vectors which express a sequence-specific DNA binding protein, comprising the steps of:

a) cloning the vector in host cells to form clonal colonies'

b) generating a replica plate of the cellular protein of the clonal colonies;

c) contacting the cellular protein with a DNA probe comprising a DNA sequence embodying the binding site of the sequence-specific binding protein under conditions which permit the sequence specific binding protein to bind the probe to form a complex; and

d) washing the cellular protein to remove unbound probe.

30. (Original) A method of claim 29, wherein the sequence-specific binding protein is a transcriptional regulatory factor.

31. (Original) A method of claim 29, wherein the expression vector is the bacteriophage \gt11.

32. (Original) A method of claim 29, wherein a nonspecific competitor DNA is contacted with cellular protein along with the DNA probe.

33. (Original) A method of claim 32, wherein the nonspecific competitor DNA is poly(dI-dC)-poly(dI-dC) or denatured calf thymus DNA.

34. (Original) A method of claim 29, wherein the probe is up to 150 bp in length.

35. (Original) A method of claim 29, wherein the probe comprises a oligomer of binding sites for the sequence-specific binding protein.

36. (Original) A labeled DNA probe complementary to at least a portion the sequence of nucleic acid encoding a transcriptional regulatory factor.

37. (Original) A DNA probe of claim 36, wherein the factor is lymphoid-specific.

38. (Original) A DNA probe of claim 37, selected from the group consisting of NF-κB and IgNF-B.

39. (Original) A method of detecting DNA or RNA encoding a transcriptional regulatory factor, comprising contacting a sample to be tested with a labeled DNA probe complementary to at least a portion the sequence of nucleic acid encoding a transcriptional regulatory factor; incubating the probe and the sample under hybridization conditions which permit the labeled probe to hybridize with complementary DNA or RNA sequences; removing unhybridized probe and analyzing the sample for hybridized probe.

40. (Original) A method of claim 39, for determination of expression of the factor, wherein the conditions of hybridization are sufficiently stringent such that the probe hybridizes only to nucleic acid sequences to which it is substantially complementary.

41. (Original) A method of claim 39, for the identification of a gene encoding a transcriptional regulatory factor, wherein the hybridization conditions are of a sufficiently relaxed stringency such that the probe will hybridize to DNA sequences which are not completely complementary.

42. (Original) Polyclonal or monoclonal antibody specifically reactive with a nuclear protein which binds, in a sequence specific manner, to a transcriptional regulatory DNA element of an immunoglobulin light chain gene, a transcriptional regulatory DNA element of an immunoglobulin heavy chain gene or both.

43. (Original) An immunoassay for detection of a transcriptional regulatory factor in a biological fluid, in which the antibody of claim 42 is used to detect the

transcriptional regulatory factor.

44. (Original) An immunoassay of claim 43, for detection of IgNF-B or NF-κB, wherein the antibody is specifically reactive with IgNF-B or NF-κB.

45. (Original) The recombinant phage \h3, ATCC 67629.

46. (Original) The recombinant phage OCT-2, ATCC 67630.

47. (Original) A method of identifying an agonist or an antagonist of gene transcription, comprising employing a gene encoding a transcriptional regulatory factor in an in vivo or in vitro assay to identify an agonist or antagonist of the factor or the gene encoding the factor.

48. (Original) An agonist or antagonist of the activity of a transcriptional regulatory factor of claim 1 or a gene encoding the factor.

49. (Original) DNA encoding the DNA binding domain of a transcriptional regulatory protein of claim 1.

50. (Original) A method of specifically stimulating gene transcription in a cell, comprising:

a) providing an expressible gene construct comprising DNA encoding the binding domain of a transcriptional regulatory factor linked to DNA encoding an activator of the RNA polymerase for the gene; and

b) introducing the construct into the cell.

51. (Original) A method of claim 50, wherein the DNA encodes the binding domain of IgNF-B or NF-κB.

52. (Original) A DNA construct comprising DNA encoding the binding domain of a transcriptional regulatory factor linked to DNA encoding an activator of the Rna polymerase of the gene.

53. (Original) A DNA construct of claim 52, wherein the DNA encodes the binding domain of IgNF-B or NF-κB.

54. (Original) A method of inducing expression of a gene, comprising the steps of:

a) preparing a DNA construct comprising:
   i) a Kappa enhancer sequence or a portion of the Kappa enhancer sequence containing at least the sequence to which the factor NF-κB binds;
   ii) a promoter; and
   iii) a structural gene of interest.
b) transfecting a eukaryotic host cell with the DNA construct; and
c) stimulating the transfected cell with a substance which stimulates NF-B activation and binding to the enhancer sequence.

55. (Original) A method of claim 54, wherein the structural gene encodes a cytotoxic protein.

56. (Original) A method of claim 54, wherein the substance which stimulates NF-B is an activator of protein kinase C.

57. (Original) A method of altering expression in a cell of a gene whose transcriptional activity is altered by binding of NF-κB to the enhancer of said gene, comprising controlling dissociation of the NF-κB-IκB complex present in the cytoplasm of said cell.

58. (Original) The method of reducing expression in a cell of a gene whose transcriptional activity is activated by binding of NF-κB to the enhancer of said gene, comprising preventing dissociation of NF-κB-IκB complex present in the cytoplasm of said cell.

59. (Original) A method of activating in a host cell an NF-κB precursor present in the cytoplasm of said host cell, the precursor comprising an NF-κB-IκB complex, comprising contacting the host cell with a substance which causes dissociation of the complex into IκB and translocation of said NF-κB into the nucleus of said cell.

60. (Original) A method of preventing activation in a host cell of an NF-κB precursor present in the cytoplasm of said host cell, the precursor comprising an NF-κB-IκB complex, comprising contacting the host cell with a substance which prevents dissociation of the complex into IκB and NF-κB.

61. (Original) A method of causing activation of an NF-κB precursor, present in the cytosol of a host cell, the NF-κB precursor being an NF-κB-IκB complex, comprising treating the cell with a substance which causes dissociation of the NF-κB complex, resulting in induction of DNA-binding activity and nuclear translocation of the NF-κB present in the complex.

62. (Original) A method of controlling expression of human immunodeficiency virus DNA in a host cell latently infected with human immunodeficiency virus DNA, comprising preventing binding of NF-κB to human immunodeficiency virus transcriptional control elements.

63. (Original) A method of claim 62 wherein binding of NF-κB to immunodeficiency virus transcriptional control elements is prevented by inhibiting dissociation of an NF-κB-IκB complex present in the cytoplasm of said host cell into IκB and NF-κB.

64. (Original) Isolated NF-κB-IκB complex.

65. (Original) Isolated DNA encoding NF-κB-IκB complex.

66. (Original) A method of regulating NF-κB-mediated gene expression in a cell, comprising altering NF-κB activity in the cell.

67. (Original) A method of regulating transduction in a cell of an extracellular signal by NF-κB, comprising altering NF-κB activity in the cell.

68. (Original) A method of claim 67 wherein NF-κB activity is reduced.

69. (Original) A method of claim 67 wherein NF-κB is enhanced.

70. (Original) A method of regulating NF-κB-mediated expression of a selected gene in a cell, comprising introducing into the cell a substance which regulates NF-κB activity in the cell.

71. (Original) A method of positively regulating NF-κB-mediated gene expression in a cell, comprising:

a) introducing into the cell a gene construct comprising a gene of interest, a DNA sequence which is the binding site of NF-κB and a promoter for the gene; and

b) maintaining the cell under conditions appropriate for expression of the gene.

72. (Original) A method of claim 71 wherein the binding site is represented by the following consensus sequence:

C C
GGGRATYYAC
T T

or equivalents thereof.

73. (Original) A method of claim 72 wherein the consensus sequence is present in the group consisting of: the Ig κ enhancer regulatory element, the SV40 enhancer regulatory element, the HIV long terminal repeat, a regulatory element of the MHC class I H2-K gene, a regulatory element of the IL-2 lymphokine gene, a regulatory element of the IL-2R gene, and a regulatory element of the interferon β PRDII gene.

74. (Original) A method of positively regulating the expression of a gene in a cell, the gene having a DNA sequence which is a binding site of NF-κB, said method comprising introducing an effective amount of NF-κB into the cell, under conditions appropriate for binding of NF-κB to the binding site.

75. (Original) A method of positively regulating in a cell the expression of a gene comprising a DNA sequence which is a binding site of NF-κB, comprising introducing into the cell a gene construct encoding NF-κB and maintaining the cell, under conditions appropriate for expression of the gene.

76. (Original) A method of positively regulating the expression

of a gene in a cell, the gene having a DNA sequence encoding a binding site of NF-κB, said method comprising inducing NF-κB activity by introducing into the cell an NF-κB inducing substance.

77. (Original) A method of claim 76 wherein the NF-κB inducing substance is selected from the group consisting of lipopolysaccharide, cyclohexamide, phorbol esters, virus, and tumor necrosis factor α phorbol myristate.

78. (Original) A method of negatively regulating the expression of a gene in a cell, the gene having a binding site of NF-κB, said method comprising introducing an inhibitor of NF-κB into the cell, under conditions appropriate for binding of the inhibitor NF-κB.

79. (Original) A method of claim 78 wherein the inhibitor of NF-κB is I-κB.

80. (Original) A method of negatively regulating the expression of a gene in a cell, the gene having a binding site of NF-κB, said method comprising introducing a gene construct encoding I-κB cell, under conditions appropriate for I-κB production and binding of I-κB to NF-κB.

81. (Original) A method of negatively regulating the expression of a gene in a cell, the gene having a binding site of NF-κB, said method comprising introducing into the cell a DNA sequence which is the binding site of NF-κB, under conditions appropriate for binding of the DNA sequence and NF-κB.

82. (Original) A method of claim 81 wherein the binding site is

represented by the following consensus of:

C C
GGGRATYYAC,
T T

or equivalents thereof.

83. (Original) A method of claim 81 wherein the consensus sequence is present in the group consisting of: the Ig κ enhancer regulatory element, the SV40 enhancer regulatory element, the HIV long terminal repeat, a regulatory element of the MHC class I H2-K gene, a regulatory element of the IL-2 lymphokine gene, a regulatory element of the IL-2R gene, and a regulatory element of the interferon β PRDII gene.

84. (Original) A method of modifying the expression of at least one gene in a cell, the gene having an NF-κB binding site, said method comprising introducing into the cell a gene construct comprising DNA encoding a modified NF-κB molecule which binds selectively to the NF-κB binding site of said selected gene or genes, under conditions appropriate for expression of the encoded modified NF-κB and binding of the modified NF-κB to the NF-κB binding site of said gene or genes.

85. (Original) A method of negatively regulating the expression of a gene in a cell, the gene having a DNA sequence encoding a binding site of NF-κB, said method comprising introducing into the cell a gene construct, the construct comprising DNA encoding a modified NF-κB molecule which comprises a DNA binding domain and lacks a RNA polymerase activating domain.

86. (Original) Isolated or recombinant IκB.

87. (Original) A composition comprising an NF-κB inhibitor.

88. (Original) A composition of claim 85 wherein the inhibitor is a peptide capable of binding NF-κB.

89. (New) A method for reducing expression in a human cell of a gene, the expression of which is inducible by an extracellular polypeptide that activates NF-kB to act as an intracellular messenger to transmit a signal that induces expression of the gene from the plasma membrane of the cell to the nucleus of the cell, which method comprises contacting the cell with a composition that diminishes the activity of NF-kB so as to thereby reduce expression of the gene in the cell.